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Application of cluster analysis and optimization to determine the synthesis conditions of macroreticular poly(styrene-*co*-divinylbenzene) microparticles with enhanced structural and adsorption properties

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Abstract

A suitable porous structure and high mechanical strength are extremely valuable properties in adsorbent polymeric microparticles. To achieve this objective, macroreticular poly(styrene-*co*-divinylbenzene) microparticles with high amounts of cross-linker and diverse proportions of porogen agent were synthesized according to the methodology of the factorial design of experiments. The structural characteristics of the microparticles and their adsorption properties of a model macromolecule (bovine serum albumin (BSA)) were studied. From the results obtained, as well as those observed in previous works [C. Garcia-Diego, J. Cuellar, Synthesis of macroporous poly(styrene-co-divinylbenzene) microparticles using *n*-heptane as the porogen: quantitative effects of the DVB concentration and the monomeric fraction on their structural characteristics, Ind. Eng. Chem. Res. 44 (22) (2005) 8237–8247; C. Garcia-Diego, J. Cuellar, Determination of the quantitative relationships between the synthesis conditions of macroporous poly(styrene-co-divinylbenzene) microparticles and the characteristics of their behaviour as adsorbents using bovine serum albumin as a model macromolecule, Ind. Eng. Chem. Res. 45 (10) (2006) 3624–3632], it was decided to apply a cluster analysis to all the adsorbents with a view to determining the range of synthesis conditions that would allow the synthesis of microparticles with high mechanical strength and a porous structure appropriate for absorbing the BSA macromolecule. This range was found to be 0.50–0.60 for the monomeric fraction and 25.0–55.0% for the divinylbenzene concentration in the monomeric mixture. Additionally, through use of the desirability function, the results revealed that the adsorbent with the optimum adsorption characteristics, as regards both adsorption equilibrium and adsorption kinetics, should be synthesized with a DVB concentration of 42.5% and a monomeric fraction of 0.50.

Keywords: Macroreticular poly(styrene-*co*-divinylbenzene) microparticles; Bovine serum albumin macromolecule; Equilibrium adsorption; Cluster analysis; Optimization; Desirability function

1. Introduction

The separation or purification of macromolecules through an adsorption process is a field that requires continuous research aimed at the development and optimization of adsorbents with properties suitable for specific applications.

An ideal adsorbent for the separation of macromolecules should have a high adsorption capacity, fast kinetics in the adsorption–desorption process, and good mechanical and chemical stability. Additionally, in order for a given molecule to be adsorbed on the surface of an adsorbent, two circumstances must be met: the chemical affinity between the molecule and the adsorbent surface, and the accessibility of the adsorption sites.

In recent years, macroreticular polymeric microparticles, in particular those based on poly(styrene-*co*-divinylbenzene), have been widely used as adsorbents owing to their high physical and chemical stability, and their permanent porous structure [1–5]. The porous structure of microparticles is due to the hollow space that is left after the removal of the inert organic diluent (porogen) added to the monomeric mixture during the polymerization reaction. Depending on the thermodynamic affinity between the diluent and the copolymer molecules, and on the proportions of diluent and cross-linker (divinylbenzene) in the organic phase, microparticles with different porous structures can be obtained [5].

Studies by Abrams and Millar [6] have determined the values of the synthesis conditions under which the onset of

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macroporosity of poly(styrene-*co*-divinylbenzene) microparticles occurs. Furthermore, in a previous work [1] we reported the synthesis of several poly(styrene-*co*-divinylbenzene) adsorbents using *n*-heptane as porogen. These adsorbents displayed different structural characteristics, which depended on their synthesis conditions. From the quantitative relationships determined in that study, which correlate the structural characteristics of the microparticles (BET specific surface area, and volume of micro-, meso- and macropores) with their synthesis conditions (weight percentage of divinylbenzene isomers in the monomeric mixture and volume fraction of the monomers in the organic phase), it was found that, in principle, the most suitable adsorbent for the adsorption of macromolecules should be synthesized with high contents of *n*-heptane, because these conditions provide the highest proportions of macropores and mesopores in the microparticles. Macropores should permit rapid diffusion of the macromolecules to the interior of each microparticle, and mesopores should provide the specific surface area for adsorption to take place. However, it was also observed that: (a) if on attempting to achieve a high proportion of macropores a high amount of diluent was used during the polymerization, microparticles with a very low mechanical strength were obtained, which caused breakage of the microparticles and hence the formation of fragments and fines, with the subsequent losses of adsorbent or operational problems such as blockages, and (b) if lower concentrations of diluent were used, microparticles with higher mechanical strength were obtained, although below a certain value for the proportion of *n*-heptane the porosity properties changed sharply because no macropores were obtained. Moreover, although the best results should be attained by using, as adsorbents, microparticles with a high proportion of macropores and mesopores, a compromise between the proportion of macropores and mesopores should be established, because macropores provide small specific surface area values and, within mesopores, the diffusion process is slow. Considering these difficulties, in this work, two aims were established. The first objective was to determine the range of conditions that would allow the synthesis of microparticles with a porous structure suitable for the adsorption of macromolecules, with a size similar to that of the macromolecule studied here, together with sufficient mechanical strength to withstand the necessary mechanical manipulations during the experimentation. The second goal was to determine, within the range of these synthesis conditions, the operating conditions which would lead to the synthesis of an adsorbent with the best compromise between its adsorption equilibrium and kinetic properties.

Here, to find a suitable porous structure for adsorbing a solute and the way of synthesizing the appropriate adsorbent for a given application, macroreticular poly(styrene-*co*-divinylbenzene) microparticles with high amounts of cross-linker were synthesized following the methodology of factorial design of experiments. The microparticles were characterized structurally using the techniques of nitrogen adsorption–desorption and mercury porosimetry, allowing us to determine the values of the specific surface areas and the pore volume of the microparticles, along with their pore size distributions. Also, mechanical strength of the microparticles was studied.

Once the porous structures of the microparticles synthesized had been characterized, experiments addressing the adsorption of a model macromolecule on these microparticles were carried out. Bovine serum albumin macromolecule was chosen as the model molecule. The adsorption isotherms allowed us to determine the maximum adsorption capacity of the macromolecule on each adsorbent by fitting the double Langmuir adsorption model to the isotherm data.

In view of the results concerning the characterization of the structural and adsorption properties of each adsorbent, and of their relative mechanical strength, we decided to apply a cluster analysis to group the adsorbents into clusters such that those in each cluster would be similar to each other and such that the adsorbents of one cluster would be different from the adsorbents of other clusters. With this clustering, a demarcation of the range of synthesis conditions that would allow the synthesis of microparticles with high mechanical strength and an appropriate porous structure for the adsorption of the bovine serum albumin macromolecule was obtained. As a final step, within this range, the process of microparticle synthesis was optimized, using the overall desirability function, in order to determine which type of adsorbent displayed enhanced adsorption properties, as regards both adsorption capacity and adsorption diffusivity.

2. Experimental

2.1. Materials

To synthesize the microparticles, styrene (St, 99%) and divinylbenzene (DVB, containing 55% DVB isomers, the remainder mainly being 3- and 4-ethylvinylbenzene) were used as monomers. Both monomers were supplied by Aldrich (Madrid, Spain). Prior to polymerization, the monomers were first washed with a 10% aqueous NaOH solution to remove the inhibitor 4-*tert*-butylcatechol and then with deionized water until neutralization. The polymerization initiator was benzoyl peroxide (BPO, 70%, remainder water), which was obtained from Aldrich (Madrid, Spain). As porogen (inert diluent), *n*-heptane (HEP, synthesis grade) was used, supplied by Panreac (Barcelona, Spain). The suspension agent, poly(vinyl alcohol) (PVA, with a weight-average molar mass of 88,000 and a degree of hydrolysis of 88%), was obtained from Acros Organics (Geel, Belgium). To wash the microparticles, acetone (reagent grade) and methanol (HPLC grade) were used. Both were supplied by Scharlau (Barcelona, Spain).

To study the properties of the microparticles as adsorbents, bovine serum albumin macromolecule (BSA, 96% purity), obtained from Sigma (Madrid, Spain), was used as a model molecule. The adsorption experiments were carried out with salt–phosphate buffer solutions, which were prepared from monobasic anhydrous sodium phosphate, dibasic anhydrous potassium phosphate and ammonium sulphate, all of them of reagent grade and all obtained from Panreac (Barcelona, Spain). The buffer solutions (pH 6.8) contained 0.05 mol/dm^3 of phosphate and 1 mol/dm^3 of ammonium sulphate.

2.2. *Synthesis of microparticles*

Macroreticular poly(styrene-*co*-divinylbenzene) microparticles were prepared with the suspension polymerization technique, as described elsewhere [1]. In a typical polymerization, a 500 mL three-necked round-bottomed jacketed glass reactor fitted with a mechanical stirrer, a condenser, and a thermometer, was loaded at 85 °C with 250 mL of an aqueous solution of 0.5 wt.% PVA. Then, an organic mixture of 50 mL of styrene, divinylbenzene, *n*-heptane, and benzoyl peroxide (0.5 wt.% of the monomers used), was poured into the reactor. Experiments with different proportions of cross-linker (DVB) and porogen (*n*-heptane) were carried out to obtain microparticles with different porous structures. Polymerizations were performed at 85 °C for 8 h at a stirring speed of 400 rpm. Following this, the microparticles obtained were washed thoroughly with hot water and vacuum-filtered to dissolve the PVA surrounding them. They were then extracted with acetone for at least 6 h in a Soxhlet apparatus to remove the porogen and residual monomers, and washed with methanol to ensure the complete removal of impurities. Finally, the microparticles were dried under vacuum in an oven at 45 °C for at least 24 h. After, they were sieved, which allowed us to determine the average value of the particle size for each adsorbent, D_p (μm). These values are given in Table 1.

2.3. *Structural and mechanical characterization of the microparticles*

Microparticles were characterized structurally using nitrogen adsorption–desorption porosimetry (Micromeritics Gemini V 2380 v1.00) and mercury porosimetry (a Carlo Erba Porosimeter 4000 instrument for adsorbents 1–8, and a Micromeritics Pore Sizer 9320 instrument for adsorbents 9–16 were used). The nitrogen adsorption data allowed us to determine the BET specific surface areas of the microparticles (S_{BET}, m^2/g) [7]. The pore volume (V_{pores}, cm^3/g, defined as the pore volume for pores with a radius between 10 and 5000 Å) and the pore size distributions of the microparticles were determined by combining the data obtained from nitrogen adsorption, following the Barret–Joyner–Halenda (BJH) method [8], and from the mercury porosimetry data.

With regard to the study of the mechanical stability of the microparticles, many tests on microparticles synthesized under

Table 1
Design matrix of the 2^2 face centred design and properties of the adsorbents

Ads. no.	Synthesis conditions				D_p (μm)	Structural and mechanical characteristics			Adsorption characteristics (BSA)[a]	
	%DVB		F_m			S_{BET} (m^2/g)[a]	V_{pores} (cm^3/g)[a]	G (g beads ≥90 μm/g beads)	q_m (mg/g dry adsorbent)[b]	D_s (cm^2/s)[c]
	Real value	Coded value	Real value	Coded value						
1	45.0	+1/3	0.55	0	144	392.2	1.011	0.9829	259.1	(1.3×10^{-10})
2	55.0	+1	0.55	0	162	447.1	1.014	0.9829	244.9	(8.5×10^{-11})
3	45.0	+1/3	0.65	+2/3	220	356.5	0.560	0.9957	37.4	(9.9×10^{-11})
4	55.0	+1	0.65	+2/3	258	413.4	0.594	0.9957	7.5	(1.1×10^{-10})
5	45.0	+1/3	0.60	+1/3	144	375.7	0.715	0.9944	170.5	(1.1×10^{-10})
6	55.0	+1	0.60	+1/3	220	439.0	0.812	0.9944	192.3	1.1×10^{-10}
7	50.0	+2/3	0.55	0	144	426.6	0.957	0.9829	244.3	(1.1×10^{-10})
8	50.0	+2/3	0.65	+2/3	202	385.3	0.605	0.9957	31.3	(1.0×10^{-10})
9	45.0	+1/3	0.55	0	170	395.1	0.883	0.9829	(259.1)	(1.3×10^{-10})
10	55.0	+1	0.55	0	144	461.8	1.087	0.9829	(244.9)	(8.5×10^{-11})
11	45.0	+1/3	0.65	+2/3	144	341.8	0.545	0.9957	(37.4)	(9.9×10^{-11})
12	55.0	+1	0.65	+2/3	144	408.7	0.576	0.9957	(7.5)	(1.1×10^{-10})
13	45.0	+1/3	0.60	+1/3	170	356.6	0.609	0.9944	(170.5)	(1.1×10^{-10})
14	55.0	+1	0.60	+1/3	144	434.8	0.810	0.9944	(192.3)	1.1×10^{-10}
15	50.0	+2/3	0.55	0	170	425.7	0.915	0.9829	(244.3)	(1.1×10^{-10})
16	50.0	+2/3	0.65	+2/3	170	369.5	0.581	0.9957	(31.3)	(1.0×10^{-10})
17	30.0	−2/3	0.40	−1	152	103.0	1.969	0.0929	134.4	1.3×10^{-10}
18	55.0	+1	0.40	−1	162	423.7	2.091	0.0929	271.0	6.0×10^{-11}
19	30.0	−2/3	0.60	+1/3	170	142.7	0.575	0.9944	150.4	1.6×10^{-10}
20	42.5	+1/6	0.50	−1/3	144	365.3	1.276	0.8899	244.8	2.0×10^{-10}
21	25.0	−1	0.50	−1/3	144	100.0	0.747	0.8899	145.4	5.6×10^{-10}
22	25.0	−1	0.70	+1	150	50.3	0.113	0.9958	8.8	4.6×10^{-10}
23	55.0	+1	0.70	+1	150	342.2	0.265	0.9958	0.0	–
24	55.0	+1	0.50	−1/3	188	426.2	1.123	0.8899	280.4	6.7×10^{-11}
25	40.0	0	0.60	+1/3	144	292.0	0.670	0.9944	168.2	5.3×10^{-11}

[a] Data for experiments 17–25 are from Refs. [1,2].

[b] Taking into account the good reproducibility of the results obtained from the structural characterization, it was considered that the values of q_m for the replicated microparticles (ads. no. 9–16, shown in brackets) would be identical to those obtained for ads. no. 1–8.

[c] The diffusivity values for microparticles whose synthesis is described here were determined by applying the model described elsewhere [2], using the coded values of %DVB and F_m obtained from the *Global coded scheme* followed in ref. [1,2]. These values are shown in brackets.

a broad range of operating conditions have been carried out at our laboratory with the purpose of ranking the microparticles as a function of their relative mechanical strengths [9]. In a typical test, 0.8 g of copolymer beads with a particle size between 90 and 150 μm were introduced, together with 16 spherical stainless-steel particles with a size of 3 mm, into a vial containing 10 mL of ethanol. Then, each vial was shaken at 1800 rpm for 2 min in a vortex shaker. After this time, microparticles were classified using a 90 μm sieve, and then dried under vacuum at 50 °C over 24 h and weighed. The particle mass fraction remaining with a particle size greater than 90 μm, G, was considered as a measure of the relative mechanical strength of the microparticles. These data were correlated through non-linear regression analysis to obtain the following sigmoidal curve with a multiple determination coefficient, R^2, of 0.930:

$$G = \frac{0.996}{1 + \exp((0.452 - F_{\mathrm{m}})/0.023)} \tag{1}$$

where F_{m} is the uncoded value of the monomeric fraction used during the synthesis of each type of adsorbent. This equation was used to quantify the relative mechanical strength of the microparticles as a function of the monomeric fraction (see values in Table 1).

Additionally, surface morphology of the microparticles was examined by scanning electron microscopy (SEM), using a Zeiss DSM 940 microscope. Some samples of beads were broken to disclose their inner structure.

2.4. *Adsorption experiments*

The operating procedure employed to obtain the adsorption equilibrium isotherms of BSA on each type of adsorbent has been described elsewhere [2]. Briefly, samples of each type of microparticles, pretreated with methanol, were placed in a column and washed with 10 column volumes of buffer. The dry-weight fraction of the wet adsorbents was determined from the weight loss of the adsorbent samples at 110 °C for 24 h in an oven. Then, amounts of wet adsorbent (0.5 g approximately) were weighed and placed in tubes containing 10 mL of the salt–buffer solution with a known initial concentration of BSA. The solution was allowed to equilibrate at 25 °C for 24 h in a rotator. After this time, the BSA concentration in the supernatant (C_{e}, mg/mL) was determined with a UV–vis spectrophotometer (Varian, Cary 50) at 280 nm. The amount of BSA adsorbed per unit dry weight of adsorbent (q_{e}, mg/g of dry adsorbent) was obtained from a mass balance.

2.5. *Planning the experiments*

The factorial design of experiments methodology [10] was followed to plan the experiments to be carried out. The synthesis variables, or factors, whose influence was studied, were the DVB concentration in the monomeric mixture (%DVB, %w/w, that is, the weight percentage of DVB isomers in the monomeric mixture) and the monomeric fraction in the organic phase (F_{m}, v/v, that is, the volume fraction of the monomers in the organic phase).

In light of the results reported in the literature [1,2], the research was planned in a range of DVB and *n*-heptane concentrations within which it would be possible to obtain microparticles with high mechanical strength and a high proportion of macro- and mesopores, and that at the same time would allow a systematic study of the region where the porous structure changes abruptly [1]. Thus, the range of values chosen for the synthesis conditions was 45.0–55.0% for the DVB concentration, and 0.55–0.65 for the monomeric fraction, and a 2^2 face centred design was selected to study this region in detail. Also, the experiments were replicated. Hence, 16 experiments were carried out. The design matrix is given in Table 1.

3. Results and discussion

3.1. *Structural and mechanical characterization of the adsorbents*

3.1.1. *Nitrogen adsorption–desorption isotherms*

The nitrogen adsorption–desorption isotherms obtained for each adsorbent synthesized are depicted in Fig. 1, where the strong similarity between the isotherms of each adsorbent and its replicate indicates high reproducibility in the synthesis of the adsorbents. It can also be observed that there are two types of isotherms. Thus, adsorbents 1, 2, 5, 6, and 7, and their corresponding replicates, display isotherms of Type II, indicating an unrestricted monolayer–multiplayer adsorption, typical of physical adsorption on macroreticular adsorbents [11]. These adsorbents show the shared feature of having been synthesized with monomeric fractions of 0.55 or 0.60. In contrast, adsorbents 3, 4 and 8, and their corresponding replicates, which were synthesized with monomeric fractions of 0.65, exhibit Type IV behaviour, typical of mesoporous materials.

The specific surface areas of each adsorbent were calculated using the BET method (Table 1), and the very similar values obtained for each adsorbent and its replicate again indicate the high reproducibility of the synthesis of the adsorbents.

3.1.2. *Mercury porosimetry*

The mercury intrusion curves obtained for each adsorbent are shown in Fig. 2. Although these curves were obtained from the mercury intrusion data collected with different instruments and operators, the high reproducibility of the synthesis of the adsorbents can be confirmed again.

It can be observed in Fig. 2 that at 200 MPa (which is the maximum pressure of mercury intrusion), the adsorbents synthesized with the highest monomeric fraction ($F_{\mathrm{m}} = 0.65$) – that is, adsorbents 3, 4 and 8, and their corresponding replicates – were penetrated by the smallest amount of mercury (below 0.4 cm^3/g). At the same pressure, the adsorbents synthesized with the smallest monomeric fraction ($F_{\mathrm{m}} = 0.55$) (that is, adsorbents 1, 2 and 7, and their corresponding replicates) were penetrated by the highest amount of mercury (more than 0.6 cm^3/g). Finally, also at the same pressure, the adsorbents synthesized with the intermediate monomeric fraction ($F_{\mathrm{m}} = 0.60$) (adsorbents 5 and 6, and their corresponding replicates) were penetrated by intermediate amounts of mercury (0.4–0.6 cm^3/g).

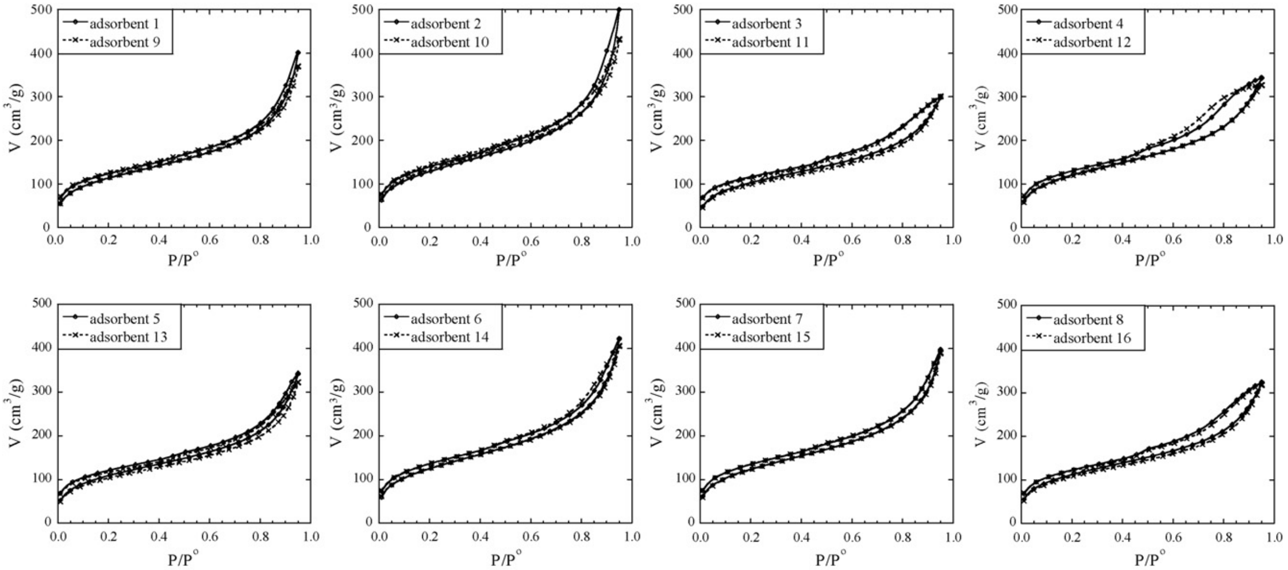

Fig. 1. Nitrogen adsorption–desorption isotherms of adsorbents 1–8, and their corresponding replicates.

From these observations, it may be inferred that the adsorbents with the highest pore volume are those synthesized with the highest *n*-heptane content (or smallest monomeric fraction) because they were penetrated by the highest amount of mercury at a given pressure.

These conclusions are consistent with those obtained from the nitrogen adsorption–desorption isotherms. In other words, the adsorbents displaying Type II isotherms are those with the highest amount of mercury intruded. In contrast, the adsorbents exhibiting Type IV isotherms are those with the smallest amount of mercury intruded.

3.1.3. *Pore size distributions*

The pore size distributions for each adsorbent, shown in Fig. 3, were obtained by combining the experimental data from their nitrogen adsorption and mercury porosimetry. Again, the high reproducibility in the synthesis of the adsorbents can be observed.

As may be seen in Fig. 3, adsorbents 3, 4 and 8, and their corresponding replicates – i.e., those synthesized with the lowest *n*-heptane content ($F_m = 0.65$) – have the lowest pore volume at any pore size and, especially, they have a very low macropore volume. With regard to the adsorbents synthesized with the

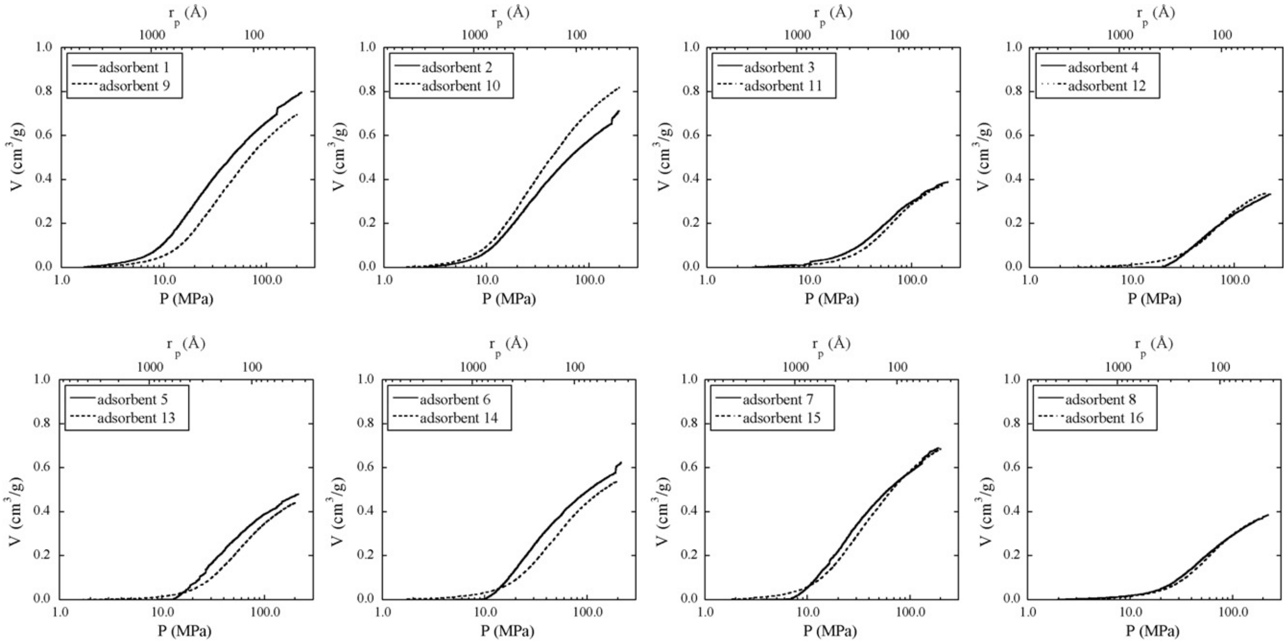

Fig. 2. Intrusion mercury curves of adsorbents 1–8, and their corresponding replicates.

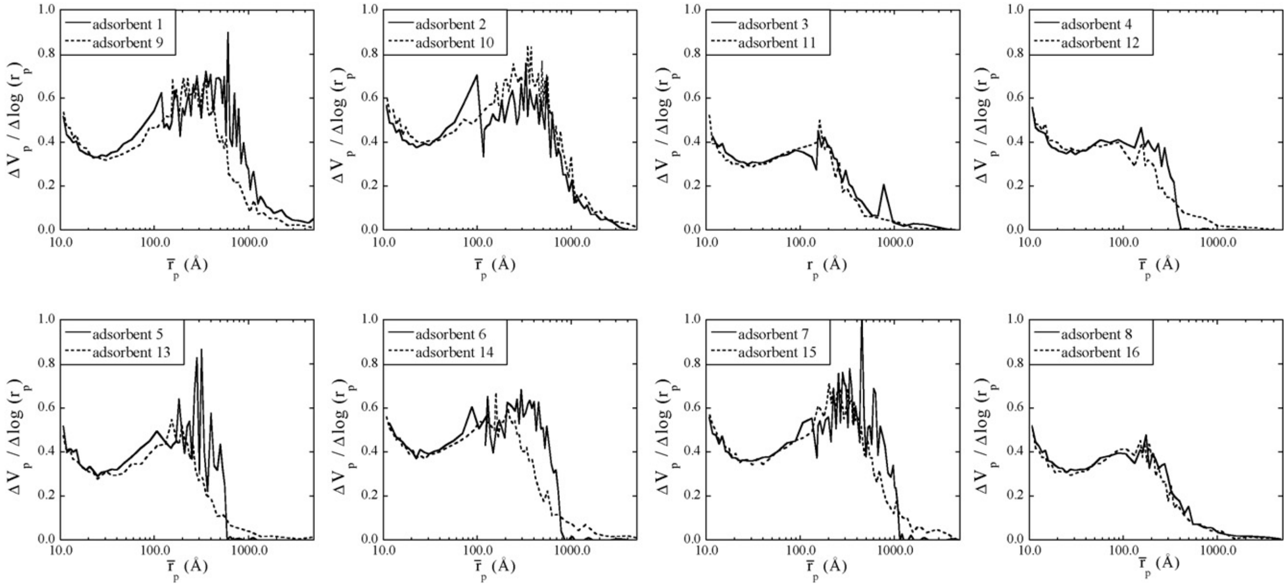

Fig. 3. Pore size distributions of adsorbents 1–8, and their corresponding replicates.

smallest monomeric fraction (adsorbents 1, 2 and 7, and their corresponding replicates), these have the highest pore volume.

3.1.4. Mechanical strength

Application of Eq. (1) allowed us to determine the relative mechanical strength of the microparticles, G (see values in Table 1). As can be observed, microparticles synthesized with a monomeric fraction of 0.40 display very low relative mechanical strength, because of the presence of very large pores. In contrast, the other microparticles have a very high relative mechanical strength (with values of G higher than 0.89), which permits experimentation with the formation of very few fragments and fines.

3.1.5. SEM photographs

Scanning electron microphotographs of the external surface and inner structure of the microparticles obtained in each experiment are shown in Fig. 4. It may be observed that the adsorbents synthesized with the highest amount of *n*-heptane ($F_m = 0.55$) – i.e., adsorbents 1 (Fig. 4a and b), 2 (Fig. 4c and d) and 7 (Fig. 4k and l) – have the most open structure, confirming the conclusions obtained from the pore size distributions of the adsorbents. With regard to adsorbents 3 (Fig. 4e and f) and 4 (Fig. 4g and h), which were synthesized with a monomeric fraction of 0.65, these have the most closed structure. The remaining adsorbents show intermediate structural characteristics.

3.2. Adsorption equilibria

The adsorption isotherms of the BSA macromolecule on only one replicate of the microparticles synthesized in this work are shown in Fig. 5. When two adsorbents have the same structural properties and chemical nature, their behaviour in an adsorption process is expected to be similar. Since in our research we observed that the values of q_m for two replicate adsorbents did not differ significantly, the isotherms for adsorbents 9–16 can be assumed to be similar to the isotherms for adsorbents 1–8. Regarding adsorbents 17–25, the adsorption isotherms of these have already been published [2].

To analyze the results, a double Langmuir equation with affinity parameter values [2] of 23.12 and 0.59 was fitted to the experimental adsorption data through multiple non-linear regression analysis. This fitting allowed us to calculate the maximum adsorption capacity of BSA on each adsorbent (Table 1). The highest adsorption capacities are obtained for adsorbents 1, 2 and 7, all of which were synthesized with a value of 0.55 for the monomeric fraction. Adsorbents 3, 4 and 8, which were synthesized with the highest monomeric fraction ($F_m = 0.65$), have the lowest adsorption capacities. Intermediate values of adsorption capacity are obtained for the microparticles synthesized with intermediate values of the monomeric fraction.

Upon comparing these results with those obtained from the structural characterization, it may be deduced that the lower the monomeric fraction (i.e., the higher amount of porogen), the higher the pore volume, and the higher the maximum adsorption capacity. These conclusions are consistent with results obtained elsewhere [1,2].

3.3. Cluster analysis

With a view to determining the range of values of the synthesis conditions that afford microparticles with high mechanical strength and good adsorption properties, the results obtained here, together with those reported in previous works [1,2], were included in the study. These results indicate that in general an increase in pore volume of the microparticles involves an increase in their adsorption capacity. It can also be deduced from the data given in Table 1 that the higher the BET specific surface area, the higher the adsorption capacity. However, for the complete range of synthesis conditions studied, it is not possible to find a satisfactory correlation between the adsorption capacity and pore volume or the BET specific surface area.

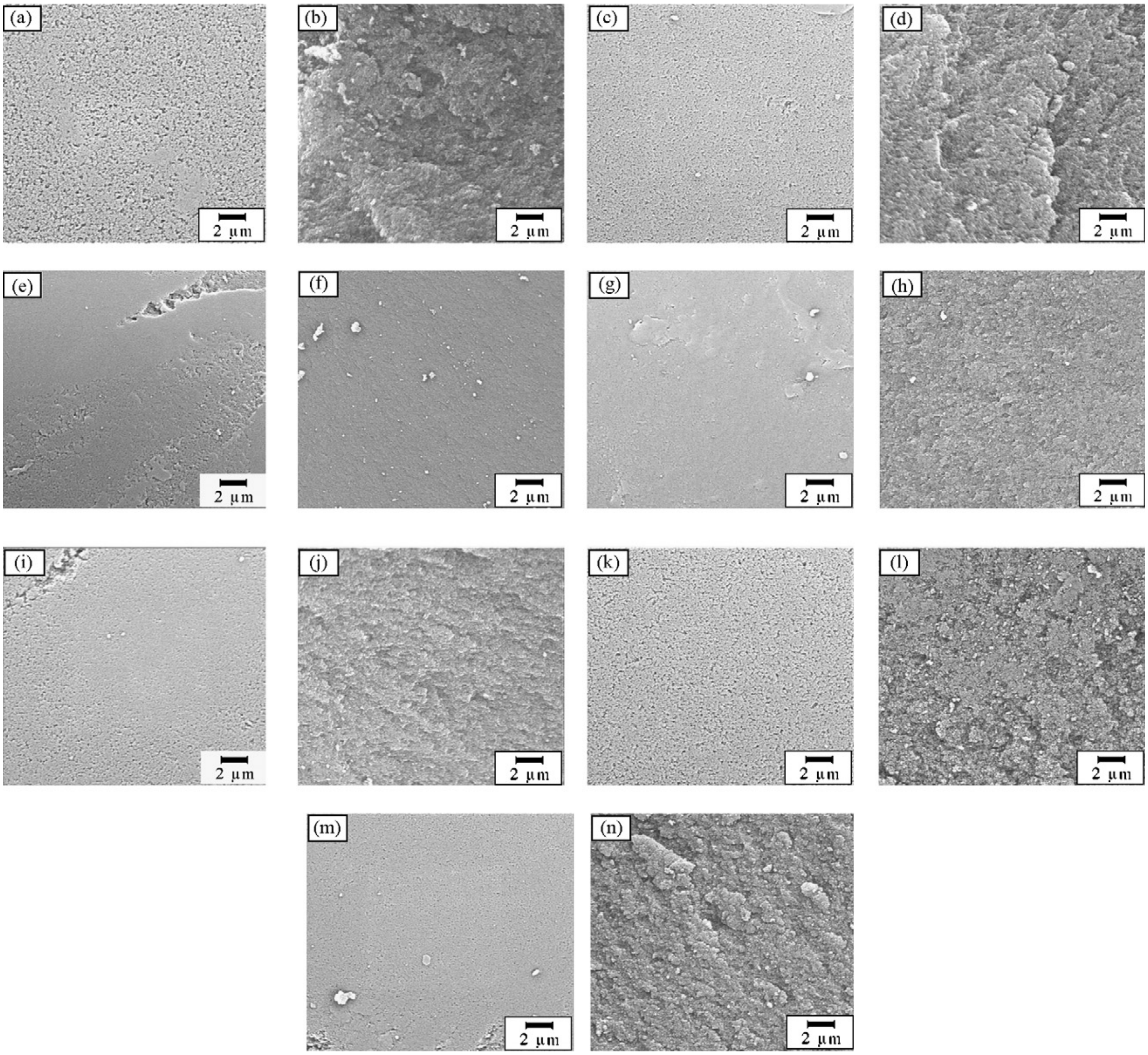

Fig. 4. Scanning electron microphotographs of the external surface and inner structure of the microparticles from: (a and b) adsorbent 1; (c and d) adsorbent 2; (e and f) adsorbent 3; (g and h) adsorbent 4; (i and j) adsorbent 5; (k and l) adsorbent 7; and (m and n) adsorbent 8. SEM photographs of adsorbent 6 can be found elsewhere [2].

However, this lack of correlation cannot be considered anomalous if the following two circumstances are taken into account. The first is that the BET specific surface area includes the surface area of the smaller pores where the macromolecule cannot penetrate and, consequently, a correlation between adsorption capacity and BET specific surface area cannot be extended to adsorbents with a high proportion of micropores. The second circumstance is that it is possible that adsorption may occur not only on the pore wall surface of the microparticles, but also that an incomplete filling of the pores can take place, especially for the macropores, and this can be the reason why a correlation between the pore volume and the adsorption capacity cannot be extended to adsorbents with a high proportion of macropores. Additionally, it has been found that there is a strong relationship between the pore volume and the mechanical strength of the microparticles (see values in Table 1), but not between the BET specific surface area and the mechanical strength.

Consequently, the pore volume (V_{pores}) and the maximum adsorption capacity (q_{m}) were chosen respectively as being representative of the structural and adsorption characteristics of the microparticles. Thus, initially a scatter plot displaying points at paired coordinates from the two variables V_{pores} and q_{m} was drawn (Fig. 6). Since it was possible to distinguish three groups of points, we decided to apply a cluster analysis to all the adsorbents in order to gather them statistically into clusters such that the adsorbents in each cluster would be similar to each other, with respect to the pair of properties considered, but different from the adsorbents of other clusters [12].

Clustering was performed with the structural and adsorption characteristics selected (V_{pores} and q_{m}), using the complete linkage method with Euclidean distances. According to this method,

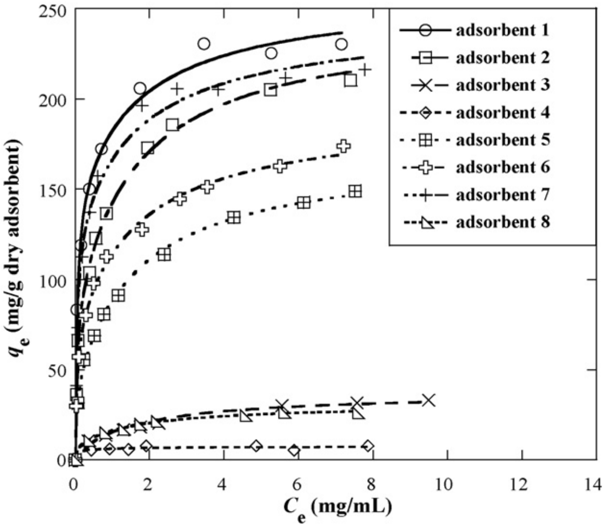

Fig. 5. Adsorption isotherms of BSA on adsorbents 1–8.

the first step involves the determination of the distances between the observations farthest away from one another for each pair of clusters. The second step is to determine which distance, of those determined in the first step, is the smallest. The pair of clusters with the smallest distance between them has the highest similarity among the observations. The following step is therefore to group both clusters in a new cluster. The amalgamation steps performed are displayed by means of a dendrogram or tree diagram (Fig. 7).

Again, with this procedure, three groups or clusters were visible. The first cluster (in the upper right corner of Fig. 6 and on the left side of Fig. 7) corresponded to microparticles synthesized with the highest content of *n*-heptane ($F_m = 0.40$). These microparticles have very low mechanical strength (see Table 1) due to the presence of very large pores inside the microparticles (pores with a radius greater than 1000 Å). The adsorbents belonging to the cluster in the lower left corner of Fig. 6 and on the right side of Fig. 7 were synthesized with the lowest amounts of *n*-heptane in the organic phase ($F_m = 0.65$ and 0.70) and have the most compact structure and the lowest BSA adsorption capacity. Indeed, it may be observed that this cluster comprises two clusters, one of them corresponding to microparticles synthesized with a monomeric fraction of 0.70 and the other corresponding to microparticles synthesized with a monomeric fraction of 0.65. In any case, both types of microparticles display Type IV nitrogen adsorption–desorption isotherms, showing the absence of macropores in them. This observation was also corroborated by their mercury intrusion curves and pore size distributions. Also, as a consequence of these structural characteristics their adsorption capacities are very low. Finally, the microparticles grouped in the remaining cluster have intermediate structural characteristics because they were synthesized at intermediate values of the operating conditions ($F_m = 0.50$–0.60).

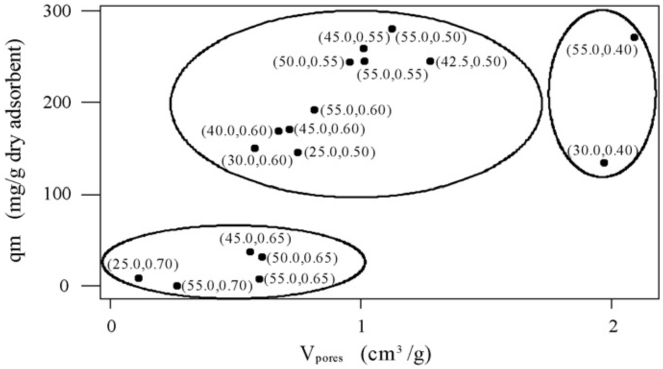

Fig. 6. Scatter plot of the polymeric microparticles. The synthesis conditions (%DVB, F_m) are shown in brackets.

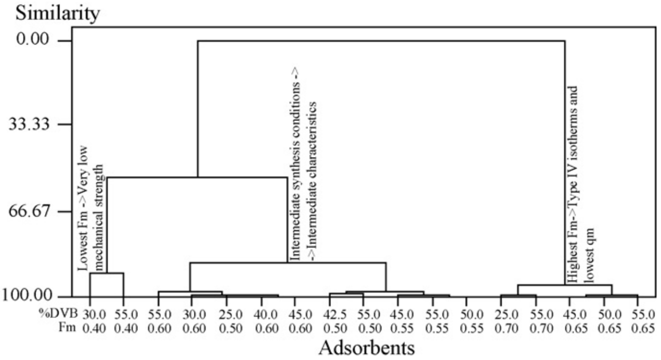

Fig. 7. Dendrogram of the polymeric microparticles.

It may thus be concluded that the range of values of the synthesis conditions that allow the generation of microparticles with high mechanical strength and a porous structure appropriate for the adsorption of the BSA macromolecule is 0.50–0.60 for the monomeric fraction and 25.0–55.0% for the divinylbenzene content.

3.4. Adsorption kinetics

Once the range of values of the synthesis conditions that allow the production of microparticles with high mechanical strength and a porous structure appropriate for the adsorption of BSA had been determined, the next step was to find, within this range (that is, adsorbents 1, 2, 5, 6, 7 19, 20, 21, 24 and 25), the adsorbent with enhanced adsorption characteristics as regards both adsorption equilibrium and adsorption kinetics. To do so, it is necessary to know previously the maximum adsorption capacities and the diffusivity values of the microparticles whose synthesis process is to be optimized. Regarding adsorbents 6, 19, 20, 21, 24 and 25, the maximum adsorption capacities (q_m) and diffusivities (D_s) of these have already been published [2]. In respect of adsorbents 1, 2, 5 and 7, the maximum BSA adsorption capacities were determined in the experiments carried out in this work and are given in Table 1. Regarding the diffusivity values of BSA in these adsorbents and the fact that these adsorbents were synthesized in the range of operating conditions within which the solid diffusion model for the adsorption of BSA on poly(styrene-*co*-divinylbenzene) microparticles (reported in a previous paper [2]) was deduced, we decided to use this model to calculate the values of this parameter from the synthesis conditions of

Table 2
Specifications for the calculation of desirability values d

	$y_1 = q_m$, mg/g		$y_2 = D_s$, cm^2/s	
Property value	225.0	175.0	2.0×10^{-10}	1.0×10^{-10}
Desirability value	0.63	0.37	0.63	0.37

each type of microparticle. The results obtained are shown in Table 1.

3.5. Optimization of the synthesis process

Once the maximum adsorption capacity and diffusivity values were known, the next step was to find the best adsorbent. Often, a high adsorption capacity is due to a large specific surface area in the adsorbent, but adsorbents with a high specific surface area usually have pores with small radii, and hence low diffusivity values. Accordingly, a compromise must be established between capacity and diffusivity. To optimize the process of the synthesis of the poly(styrene-*co*-divinylbenzene) microparticles, the overall desirability function, D, was used [13]. This function deals with a dimensionless desirability scale, or the d scale, to which the physical properties (maximum adsorption capacity and diffusivity) are transformed so that they can be interpreted in terms of utility for applications of the microparticles as adsorbents. Although many scales are possible, a useful one ranges between 0.00 and 1.00, such that $d = 0.00$ corresponds to a completely unwanted level of the property, and $d = 1.00$ corresponds to a completely acceptable level of the property, i.e., an improvement would serve no useful purpose. Additionally, two useful landmarks should be given: (a) a value of $d = 0.37$, which is approximately $1/e$, where e is the base of the natural logarithms, is assigned to the minimum acceptable value of the property; and (b) a value of $d = 0.63$, which is approximately $1 - 1/e$, corresponds to an acceptably good value of the property.

Since it is required that the maximum adsorption capacity and diffusivity should be as high as possible, there is no upper specification limit, only a lower one. In this case, the following exponential relationship should be used to transform the values of the physical property, y, into d:

$$d = \exp[-\exp(-y')] \tag{2}$$

where y' is given by:

$$y' = b_0 + b_1 y \tag{3}$$

The two constants, b_0 and b_1, are calculated by assigning the corresponding values of d to two values of y, as shown in Table 2. These values allowed us to determine expressions for the desirability values of d_1 and d_2 as follows:

$$d_1 = \exp[-\exp(2.676 - 0.015 y_1)] \tag{4}$$

$$d_2 = \exp[-\exp(0.761 - 7.7 \times 10^9 y_2)] \tag{5}$$

Once both physical properties were transformed to the dimensionless scale of d (Table 3), it was possible to combine these d's by their geometric mean to measure the overall desirability, D. Thus, taking into account Eqs. (4) and (5), the value of D was calculated by the following equation:

$$D = \exp\left\{-\frac{1}{2}[\exp(2.676 - 0.015 y_1) + \exp(0.761 - 7.7 \times 10^9 y_2)]\right\} \tag{6}$$

Table 3
Specifications for the desirability values of d and D

	Ads. no.									
	1	2	5	6	7	19	20	21	24	25
d_1	0.742	0.692	0.324	0.444	0.689	0.218	0.691	0.194	0.805	0.312
d_2	0.455	0.329	0.400	0.400	0.400	0.536	0.632	0.972	0.279	0.241
D	0.581	0.477	0.360	0.421	0.525	0.342	0.661	0.434	0.474	0.274

In Table 3, it can be observed that the microparticles synthesized with a DVB concentration of 42.5% and a monomeric fraction of 0.50, corresponding to those synthesized in experiment 20, have a maximum value of D equal to 0.661, i.e., these microparticles should be considered the best candidates for the solute-adsorbent system studied.

4. Conclusions

Here, a contribution towards the eventual synthesis of tailor-made adsorbents has been made. Thus, the range of synthesis conditions of polymeric microparticles with a porous structure suitable for the adsorption of the BSA macromolecule, or other macromolecules of similar size, together with high mechanical strength was determined. Also, the process of the synthesis of the adsorbents was optimized within this range of operating conditions.

For this purpose, macroreticular poly(styrene-*co*-divinylbenzene) microparticles with high amounts of cross-linker were synthesized. The microparticles obtained were characterized structurally using the following techniques: nitrogen adsorption–desorption porosimetry, mercury porosimetry and SEM. The adsorption capacities of the BSA macromolecule on the microparticles were also determined, with the finding that the highest adsorption capacities are obtained when the microparticles are synthesized with the lowest monomeric fraction. The results obtained for these microparticles as well as those determined for microparticles synthesized under other different synthesis conditions [1,2], allowed us to apply a cluster analysis to all the adsorbents in order to determine the range of synthesis conditions of polymeric microparticles with a porous structure suitable for the adsorption of the BSA macromolecule together with high mechanical strength. It was found that the most suitable range is 0.50–0.60 for the monomeric fraction and 25.0–55.0% for the DVB concentration. Additionally, within this range, the process of the synthesis of the microparticles was optimized, observing that the microparticles synthesized with a DVB concentration of 42.5% and a monomeric fraction of 0.50 display enhanced adsorption properties in terms of their application in possible derivatization or as adsorbents.

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